Supporting Information

Food polyelectrolytes compress the colonic mucus hydrogel by a Donnan mechanism

*Asher Preska Steinberg[1], Zhen-Gang Wang[1], and Rustem F. Ismagilov[1,2]**

[1]*Division of Chemistry and Chemical Engineering*

[2]*Division of Biology and Biological Engineering*

California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125, United States

Derivation of the ionic contribution to osmotic pressure due to Donnan partitioning. We imagine a negatively charged polyelectrolyte solution with added salt to be in contact with the mucus layer. We take the volume of the polyelectrolyte solution (V_P) to be much larger than that of the mucus layer (V_M), which is true in our *ex vivo* set-up. In our *ex vivo* experiments, the polyelectrolyte solution volume is $V_P \sim 200\ \mu L$, and we can estimate V_M using the average thickness of colonic mucus measured in ref. [1] ($t \sim 70\ \mu m$) and the *xy* dimensions of the explants (~1 by 1 *cm*), which gives $V_M \sim 7\ \mu L$. Therefore, $\frac{V_P}{V_M} \sim 30$, and we can assume that the salt and polyelectrolyte concentrations in the polyelectrolyte solution are unaffected by any partitioning of ions into the mucus layer.

The total concentration of salt cations in the polyelectrolyte solution from the condition of electroneutrality is simply (assuming the counterion of the polyelectrolyte is the same as the cation from salt, which is the case in our system):

$$c_+^p = c_0 + p$$

(S1)

Where c_+^p is the total concentration of salt cations in the polyelectrolyte solution phase, c_0 is the salt concentration, and p is the charge concentration from the polyelectrolyte backbones. The concentration of the salt anions (c_-^p) is just:

$$c_-^p = c_0$$

(S2)

This gives an osmotic pressure due to the small ions in the polyelectrolyte solution phase as:

$$\Pi_{ion}^{P} = RT(2c_0 + p)$$

(S3)

where $R = N_{avo}k$ is the gas constant.

Now consider the small ion concentrations in the mucus layer. The mucus network contributes a fixed polyelectrolyte charge density of m. Electroneutrality then dictates:

$$c_+^m = c_-^m + m$$

(S4)

where c_+^m and c_-^m are the small cation and small anion concentrations, respectively. Let ψ be the potential difference between the mucus layer and the polyelectrolyte solution, then equality of electrochemical potential for the small ions entails:[2]

$$e\psi + RTlnc_+^m = RTlnc_+^p$$

(S5)

$$-e\psi + RTlnc_-^m = RTlnc_-^p$$

(S6)

Eq S5 and S6 can be combined to give:

$$c_+^m c_-^m = c_+^p c_-^p$$

(S7)

Combining eq S1, S2, S4, and S7 then gives:

$$c_+^m = \frac{1}{2}\left[\sqrt{m^2 + 4c_0(c_0 + p)} + m\right]$$

(S8)

and:

$$c_-^m = \frac{1}{2}\left[\sqrt{m^2 + 4c_0(c_0 + p)} - m\right]$$

(S9)

The osmotic pressure from the small ions in the mucus layer is thus:

$$\Pi_{ion}^m = RT\sqrt{m^2 + 4c_0(c_0 + p)}$$

(S10)

The osmotic pressure difference between the polyelectrolyte solution and the mucus layer due to ions ($\Delta\Pi_{ion}$) is obtained by subtracting eq S10 from eq S3:

$$\Delta\Pi_{ion} = RT\left[2c_0 + p - \sqrt{m^2 + 4c_0(c_0 + p)}\right]$$

(S11)

In the limit of $m \ll c_0$, the expression simplifies to:

$$\Delta\Pi_{ion} = RT\left[2c_0 + p - 2\sqrt{c_0(c_0 + p)}\right]$$

(S12)

Estimation of the compression modulus for the colonic mucus hydrogel. The simplest model for uniaxial deformations of a polymer network can be derived from the "affine network model", which assumes affine deformation of the polymer network. The driving physics behind deformations in this model is the entropic elasticity of the chains.[3] This model gives the classical stress-elongation relation as (also eq 8 in main text):

$$\sigma_{eng} = -G(\lambda - \frac{1}{\lambda^2})$$

(S13)

Where σ_{eng} is the engineering stress or the applied stress on the network (which in this case we took to be $\Delta\Pi$), G is the compression modulus of the network (in Pa), and λ is the deformation factor. The negative sign in front of G is due to the fact that we are applying a compressive stress. In this model, G can be written as:

$$G = \frac{\rho RT}{M_s}$$

(S14)

where ρ is the mass concentration of network strands (kg/m^3) and M_s is the MW of a network strand (in kDa). If we take the MW of a MUC2 network strand to be the MW of the polymer between network cross-links (often referred to as a "MUC2 monomer" in the biology literature), we can estimate $M_s \sim 400 - 600\ kDa$.[4,5] There are not existing literature values for the mass concentration of the murine colonic mucus hydrogel, but for porcine gastrointestinal mucus it is:

$\rho \sim 19 - 30\ mg/mL$.[6,7] Taking the arithmetic mean of these values and inserting them into eq S14 yields $G \sim 120\ Pa$. We speculate that eq S14 may be lower than the value for *G* obtained by the curve fitting done in Figure 3 because eq S14 assumes that the network strands are non-interacting.

References

(1) Datta, S. S.; Preska Steinberg, A.; Ismagilov, R. F. Polymers in the Gut Compress the Colonic Mucus Hydrogel. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (26), 7041–7046. https://doi.org/10.1073/pnas.1602789113.

(2) Overbeek, J. T. G. The Donnan Equilibrium. *Prog. Biophys. Biophys. Chem.* **1956**, *6*, 58–64.

(3) Rubinstein, M.; Colby, R. H. *Polymer Physics*; OUP Oxford: New York, 2003.

(4) Ambort, D.; Johansson, M. E. V.; Gustafsson, J. K.; Nilsson, H. E.; Ermund, a.; Johansson, B. R.; Koeck, P. J. B.; Hebert, H.; Hansson, G. C. Calcium and PH-Dependent Packing and Release of the Gel-Forming MUC2 Mucin. *Proc. Natl. Acad. Sci.* **2012**, *109* (15), 5645–5650. https://doi.org/10.1073/pnas.1120269109.

(5) Axelsson, M. A. B.; Asker, N.; Hansson, G. C. O-Glycosylated MUC2 Monomer and Dimer from LS 174T Cells Are Water- Soluble, Whereas Larger MUC2 Species Formed Early during Biosynthesis Are Insoluble and Contain Nonreducible Intermolecular Bonds. *J. Biol. Chem.* **1998**, *273* (30), 18864–18870. https://doi.org/10.1074/jbc.273.30.18864.

(6) Sellers, L. A.; Allen, A.; Morris, E. R.; Ross-Murphy, S. B. The Rheology of Pig Small

Intestinal and Colonic Mucus: Weakening of Gel Structure by Non-Mucin Components. *BBA - Gen. Subj.* **1991**, *1115* (2), 174–179. https://doi.org/10.1016/0304-4165(91)90027-E.

(7) Georgiades, P.; Pudney, P. D.; Thornton, D. J.; Waigh, T. A. Particle Tracking Microrheology of Purified Gastrointestinal Mucins. *Biopolymers* **2014**, *101* (4), 366–377. https://doi.org/10.1002/bip.22372.

(8) Hoogendam, C. W.; De Keizer, A.; Cohen Stuart, M. A.; Bijsterbosch, B. H.; Smit, J. A. M.; Van Dijk, J. A. P. P.; Van Der Horst, P. M.; Batelaan, J. G. Persistence Length of Carboxymethyl Cellulose as Evaluated from Size Exclusion Chromatography and Potentiometric Titrations. *Macromolecules* **1998**, *31* (18), 6297–6309. https://doi.org/10.1021/ma971032i.

(9) Sjöholm, E. Size Exclusion Chromatography of Cellulose and Cellulose Derivatives. In *Handbook of Size Exclusion Chromatography and Related Techniques;* Marcel Dekker: 2004, 331–352.

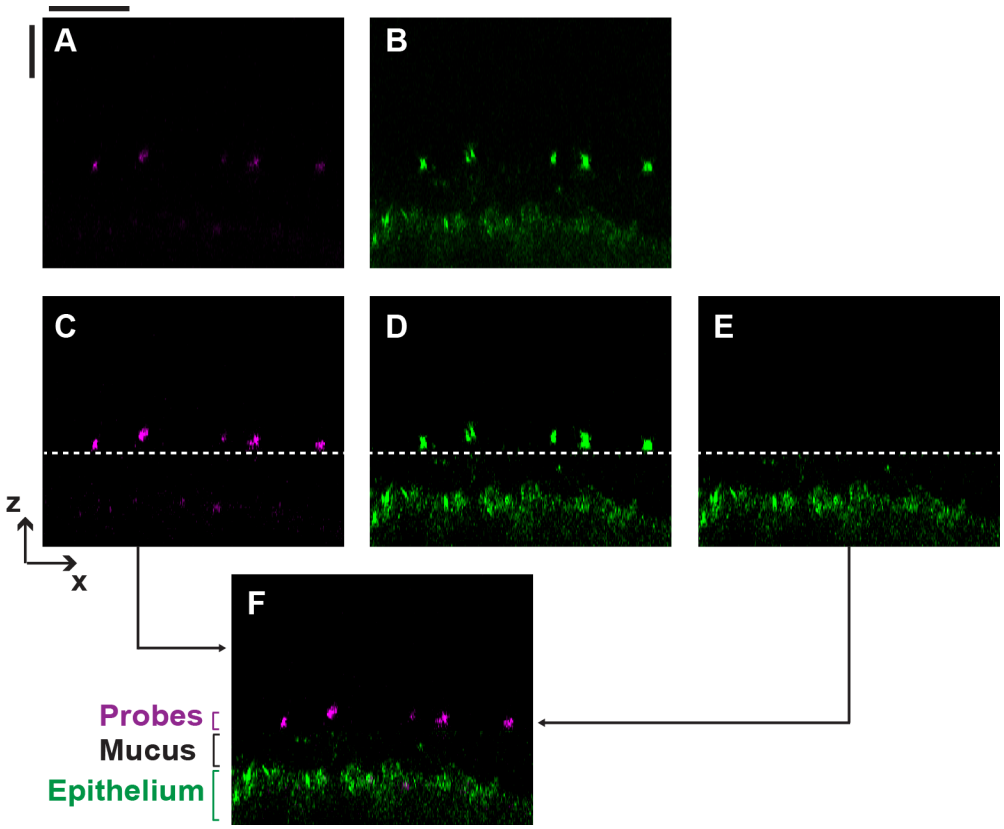

Figure S1. Description of image processing for side-views presented in Figure 2. (A-B) False-colored confocal fluorescence (*A*) and confocal reflectance (*B*) xz side-views presented in Figure 2B. Brightness and contrast was not enhanced from the original images in either panel. (C) The confocal fluorescence image in *A* but with enhanced brightness and contrast. (D) The confocal reflectance image in (*B*) but with enhanced brightness and contrast. (E) The confocal reflectance image from *D* but with the top part of the image, above the dashed line, removed. Because the particles also scatter light, we split the image below the position of the particles, which were located in the fluorescence image (shown in *C*) for clarity. The dashed line in *C*, *D*, and *E* are at the exact same z-position (right below the particles). (F) Combination of *C* and *E* presented in Figure 2B. Scale bars are 30 μm.

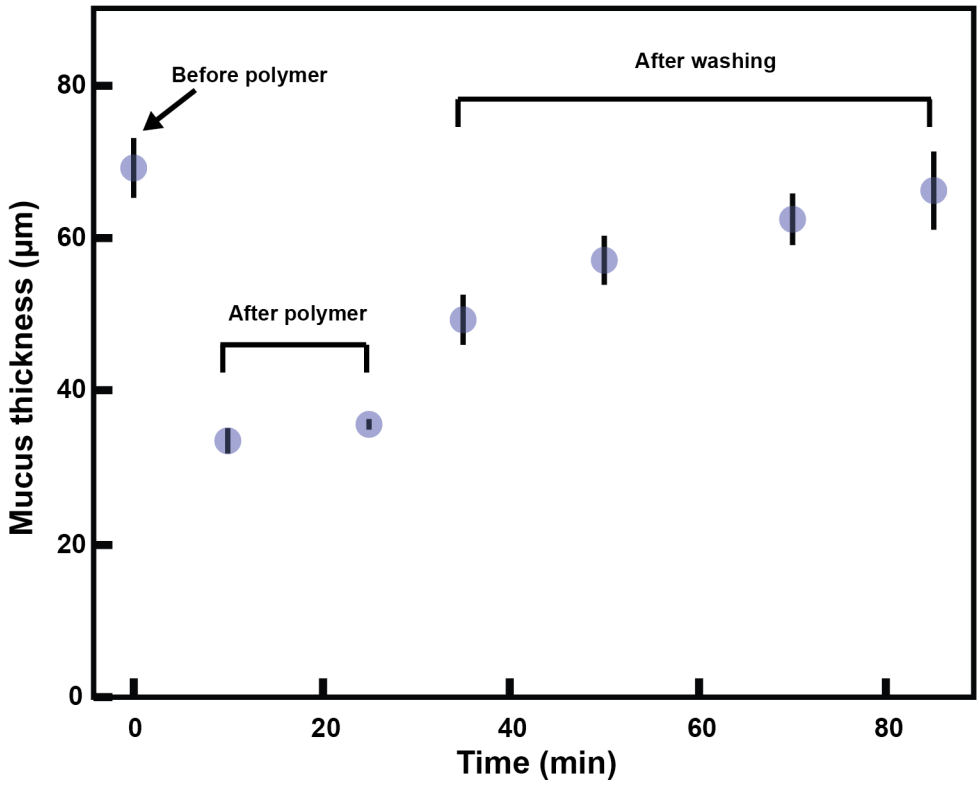

Figure S2. Compression with carboxymethyl cellulose (CMC) is reversible. Plot of mucus thickness over time before and after adding CMC with a degree of substitution of 0.7 to a murine colonic explant. The following time-points were taken: Before adding CMC (time = 0 min), 10 and 25 min after adding CMC (time = 10 and 25 min), and then 10 min to an hour after washing the explant three times with 1 mL of ice-cold 1x PBS to remove the CMC from the explant (time = 35 to 85 min). Mucus thickness was measured using the "microparticle method" (see *Materials and Methods)* and each data point represents the average thickness measured at 5 points on the explant. Error bars are SEM with n = 5.

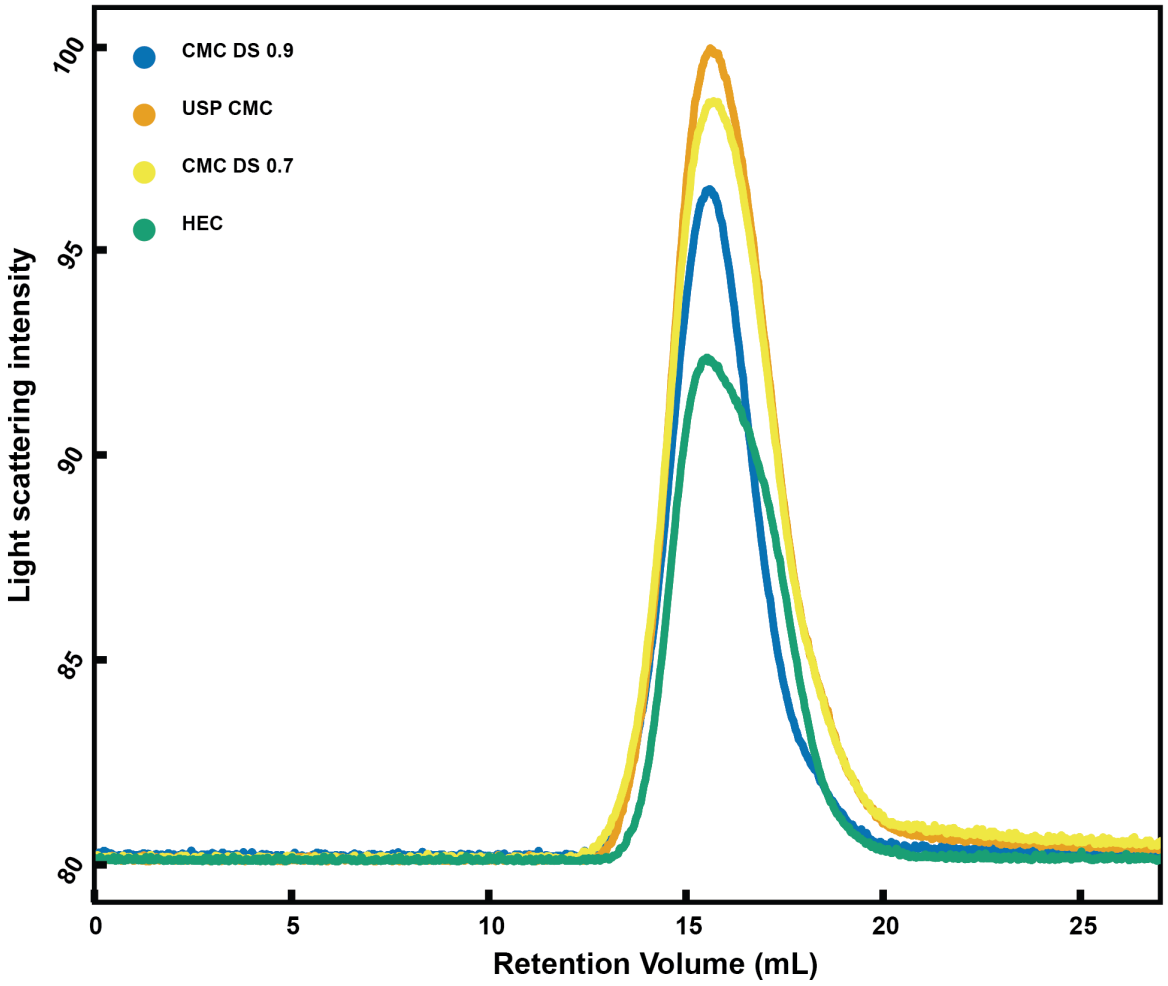

Figure S3. Gel permeation chromatography (GPC) measurements of charged and uncharged polymers. Chromatograms of polymers used in the study. Method of detection is right-angle light scattering which is plotted on the vertical axis (unitless). CMC DS 0.9 = carboxymethyl cellulose with a degree of substitution of 0.9, USP CMC = U.S.P. grade carboxymethyl cellulose fed to mice in Figure 1, CMC DS 0.7 = carboxymethyl cellulose with a degree of substitution of 0.7, HEC = hydroxyethyl cellulose.

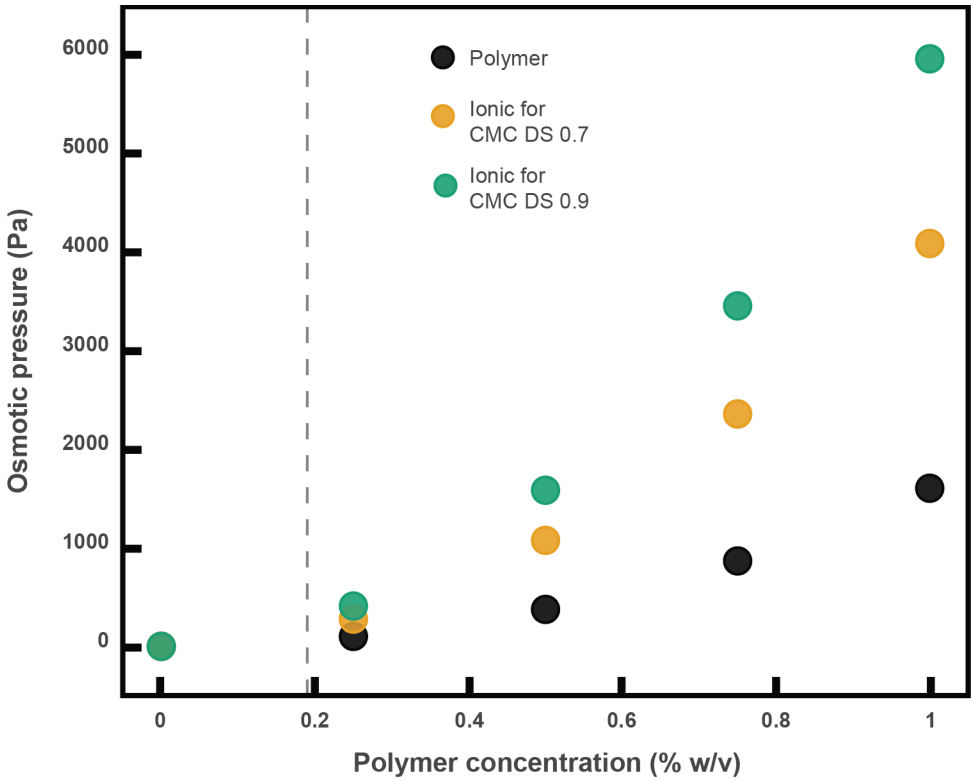

Figure S4. Polymer contribution and ionic contribution to the osmotic pressure. The contributions to the osmotic pressure (eq 4) from ionic effects (i.e. Donnan partitioning) which is given by eq 5 and from the polymer osmotic pressure which is given by eq 6. The polymer osmotic pressure (black) is equal for all polymers (both carboxymethyl cellulose [CMC] derivatives and hydroxyethyl cellulose [HEC]). There is no ionic contribution for HEC as it is uncharged. Dashed line indicates the polymer overlap concentration (c^*), where $c^* = 0.19\ \%w/v$. "Ionic for CMC DS 0.7" is the ionic contribution to the osmotic pressure for carboxymethyl cellulose with a degree of substitution of 0.7. "Ionic for CMC DS 0.9" is the ionic contribution to the osmotic pressure for CMC with a degree of substitution of 0.9.

Table S1: Gel permeation chromatography of polymers in phosphate-buffered saline.

Sample	HEC	USP CMC	CMC DS 0.9	CMC DS 0.7
M_w (kDa)	152	148	150	146
M_w/M_n	3.17	2.19	2.25	2.10
R_h (nm)	18.8	20.6	22.2	19.9

Carboxymethyl cellulose derivatives were analyzed using a refractive index increment (dn/dc) of $\frac{dn}{dc} = 0.163$.[8] Hydroxyethyl cellulose was analyzed using $\frac{dn}{dc} = 0.150$.[9] HEC = hydroxyethyl cellulose, USP CMC = U.S.P. grade carboxymethyl cellulose (fed to mice in Figure 1), CMC DS 0.9 = carboxymethyl cellulose with a degree of substitution of 0.9, CMC DS 0.7 = carboxymethyl cellulose with a degree of substitution of 0.7. M_w = weight-average molecular weight; M_w/M_n = the dispersity; R_h = hydrodynamic radius.